Studies on plant cancers—I. The mechanism of the formation of the leafy crown gall*

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(WITH PLATES 17 AND 18)

Smith in 1916 (1) announced a new type of crown gall, consisting of leafy shoots, which was produced by inoculating the leaf axils of a plant, where a dormant bud was present, with *Bacterium tumefaciens*. Similar leafy crown galls were produced by inoculating the midvein of the leaf of the tobacco, with the bacterial organism. Smith considered this type of crown gall identical with the atypical teratoid embryomata found in the animal. In 1917 (2) he showed further evidence of the power of this organism to produce leafy shoots in fifteen different families of plants. He contends that the leafy tumor is produced by inoculating *Bacterium tumefaciens* into the tissue of a susceptible species in the vicinity of totipotent cells.

Levin and Levine in 1918 (3) indicated that these leafy shoots are always secondary and that the crown gall develops first and then a group of crown gall cells become differentiated and give rise to a tissue, an organ, or potentially an entire plant, the leafy shoot. According to these authors, such differentiation of cells of a malignant tumor does not occur in animal cancer. Crown gall represents only one type in the large group of pathological processes known under the general term "cancer."

It occurred to the writer that if, as Smith claims, *Bacterium tumefaciens* inoculated into the epidermis (epithelium) of a plant gives rise to an epithelioma and a similar inoculation into the cortex or vascular bundles (connective tissue) produces a sarcoma, then the inoculation of a plant in any region of totipotent cells (bud Anlage), which are known to produce leafy shoots under normal conditions should produce them under the added stimulus of *Bacterium tumefaciens* much more readily and in greater abundance.

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It is well known that when *Bryophyllum calycinum* leaves are detached from the mother plant and are put on moist soil the marginal notches of the leaves, at which totipotent cells are found, develop into leafy shoots and eventually form new plants. For this reason, leaves and stems of *B. calycinum* were used to study the effect of the bacterium on the leafy shoot formation.

METHOD AND MATERIAL

The leaves of *B. calycinum* were detached from the plant and were placed on moist soil in pots in the greenhouse. The marginal notches of one side of the leaf, right or left, upper or lower, were inoculated, by pricking the tissue five to ten times with a delicate needle containing a culture of *Bacterium tumefaciens* from five to forty days old. The uninoculated notches of the opposite side of the leaf, served as controls, after they had been pricked with a sterile needle. Entire leaves with each notch pricked five to ten times with a sterile needle were also used as controls. Leaves in all stages of development were used. The veins of leaves and growing regions of stems of the *Bryophyllum* were also inoculated with *Bacterium tumefaciens*. In all over a thousand inoculations were made. It may be stated that thin young leaves did not lend themselves to these experiments, because they dried out too rapidly. It was likewise found that normal embryos develop better in the greenhouse than in the open.

As a rule, two days after inoculation with *Bacterium tumefaciens* both the infected and control notches showed necrotic areas in those regions. It appeared, however, that the wounds infected showed greater areas of dead tissue, which subsequently caused deeper indentations at the margin of the leaf. This was also observed by Levin and Levine (3) for a number of plants. The uninfected or control notches recovered readily and although slight scars were formed, the bud Anlagen in the notches developed into normal embryos forty to seventy days after injury. Inoculations were made: (1) into the notches of *Bryophyllum calycinum* leaves; (2) into the leaf in the vicinity of the notches; (3) into the midveins of the leaf; and (4) into the growing regions of the young stems of this plant.

OBSERVATIONS

1. *Inoculation of Bacterium tumefaciens into the notches of Bryophyllum calycinum leaves*

The study of this material shows that in the great majority of cases, the notches infected with *Bacterium tumefaciens*, instead of causing the development of leafy shoots, formed ordinary crown galls. FIG. 1 represents a young detached leaf forty-five days after having been placed on soil and having had the basal notches inoculated with the bacterium. The apical notches were pricked with a sterile needle and served as controls. The inoculated notches show well-developed ordinary crown galls without leafy shoots, while the control notches on the ventral surface show the beginning of the development of leafy shoots.

FIG. 2 represents an older leaf, in which the basal notches were inoculated forty-five days previously. In this case no galls or shoots have as yet been formed at the notches but the control notches are beginning to proliferate and the uppermost notch of the leaf has produced a shoot. FIGS. 3 and 4 represent the ventral and dorsal surfaces of a detached leaf seventy days after inoculating the basal notches with the bacterium. All the basal, infected notches show well-developed galls without leafy shoots on the ventral surface (FIG. 3). The majority of the control, apical notches have already developed leafy shoots. FIG. 5 represents an old detached leaf grown on soil, 140 days after the basal notches were infected. The mother leaf is seen in the center of the figure with a number of well-developed shoots coming from the apical notches. The basal, inoculated notches all show well-developed crown galls. In one instance a poorly developed shoot, visible in FIG. 5 over the largest gall, made its appearance. A similar condition is shown in FIG. 7. These leafy shoots appeared much later in the development of the gall. This seems to show the dwarfing and inhibiting effect the crown gall organism has on the growth of the bud Anlage.

2. *Inoculation in the vicinity of the notch*

When the inoculation is made near the notch instead of in it, a crown gall is developed alongside of a poorly developed leafy

shoot. This is shown in FIG. 6, which represents a leaf forty-five days after inoculation with *Bacterium tumefaciens*. The control notch developed a much larger and more vigorous leafy shoot.

FIG. 7 represents an old detached leaf, 140 days after inoculation. Here again several of the inoculations were made near the bud Anlagen. To the left are seen the large plants which have developed from the control notches. At the right, in the foreground, are seen three galls; next to the lower ones there appear small dwarfed plants. It appears that the galls have interfered with the normal development of these leafy shoots when compared with the large normal plants seen to the left.

3. *Inoculation in the midvein*

It may be assumed that the midvein may have totipotent cells which by the inoculation of *Bacterium tumefaciens* can be stimulated to develop leafy shoots. Forty leaves of *B. calycinum*, both young and old, detached from and attached to the mother plants, were inoculated with *Bacterium tumefaciens* by means of pricking the midvein of the leaf with a fine needle. All produced crown galls within a month after the inoculation. FIGS. 2 and 3 show the appearance of such galls on young leaves forty days after the inoculations were made. FIG. 8 represents an old leaf attached to the mother plant. A large gall has been formed on the midvein by inoculating it with the bacterium five months previously. The tumor is a charcteristic crown gall consisting of a great number of tubercles. This leaf was carefully guarded in the hopes that these tubercles would produce leafy shoots. FIG. 9 represents the gall shown in FIG. 8, nine months after inoculation. The leaf became detached and withered. The gall has grown considerably larger, taking on a cylindrical shape, and has become covered with numerous tuberosities. No leafy shoots were formed.

4. *Inoculation of the growing region of the stem*

A large number of *B. calycinum* plants were also inoculated with *Bacterium tumefaciens* in the growing region of the stem with the object of stimulating there the possible totipotent cells to leafy shoot formation. FIG. 10 (*a*, *b*, *c*, *e*, *f*) represents a few of the

young plants in which the growing regions had been inoculated four months previously. In one case only has a small leafy shoot been formed (FIG. 10, *b*). This, however, appeared after the crown gall had been well established. The plants are all dwarfed, as may be seen by comparing FIG. 10, *a*, *b*, *c*, *e*, and *f*, with *d*, one of the control plants.

SUMMARY AND CONCLUSIONS

1. *Bacterium tumefaciens* inoculated by pricks of a delicate needle into the marginal notches of a leaf of *Bryophyllum calycinum*, where totipotent cells are present, results in the formation of a crown gall as readily as in other plants used for inoculation but without leafy shoots.

2. Inoculation of *Bacterium tumefaciens* into the tissue of a leaf of *B. calycinum* in the vicinity of a small bud causes the formation of a gall and interferes with the normal development of the bud or leafy shoot.

3. Inoculation of *Bacterium tumefaciens* into the midvein of a young or old leaf detached from or attached to the mother plant results in the development of a large gall without the development of leafy shoots.

4. Inoculation of *Bacterium tumefaciens* into the growing region of the stem of a young plant causes the development of the ordinary crown gall with the occasional and subsequent development of a leafy shoot.

5. *Bacterium tumefaciens* does not cause the formation of leafy shoots in *Bryophyllum calycinum* but rather inhibits and retards their normal development, when inoculated into the totipotent cells which appear at the notches of the leaf.

LITERATURE CITED

1. **Smith, E. F.** Crowngall studies showing changes in plant structures due to a changed stimulus. Jour. Agr. Research **6**: 179–182. *pl. 18–23*. 1916.

2. —— Embryomas in plants. (Produced by bacterial inoculations.) The John Hopkins Hospital Bull. **28**: 277–294. *pl. 26–53*. 1917.

3. **Levin, I., & Levine, M.** Malignancy of the crown gall and its analogy to animal cancer. Proc. Soc. Exp. Biol. and Med. **16**: 21–22. 1918.

Description of plates 17 and 18

PLATE 17

FIG. 1. Young leaf of *Bryophyllum calycinum*, showing well-developed crown galls at the basal notches, inoculated with *Bacterium tumefaciens* forty-five days previously.

FIG. 2. Older leaf, control notches showing leafy shoots, none appearing at the inoculated notches forty-five days later. The midvein, also inoculated, shows ordinary crown gall.

FIGS. 3, 4. Ventral and dorsal surfaces of a leaf, the basal notches and midvein of which were inoculated with *Bacterium tumefaciens*, showing well-developed galls without leafy shoots. The control, apical notches show normal leafy shoots.

FIG. 6. Young leaf, in which the inoculations were made near the apical notches, showing a dwarfed leafy shoot with a crown gall attached to it. One of the control notches shows a well developed leafy shoot.

FIG. 10. Young plants. In *a*, *b*, *c*, *e*, and *f*, the growing regions were inoculated; in *d*, there was no inoculation.

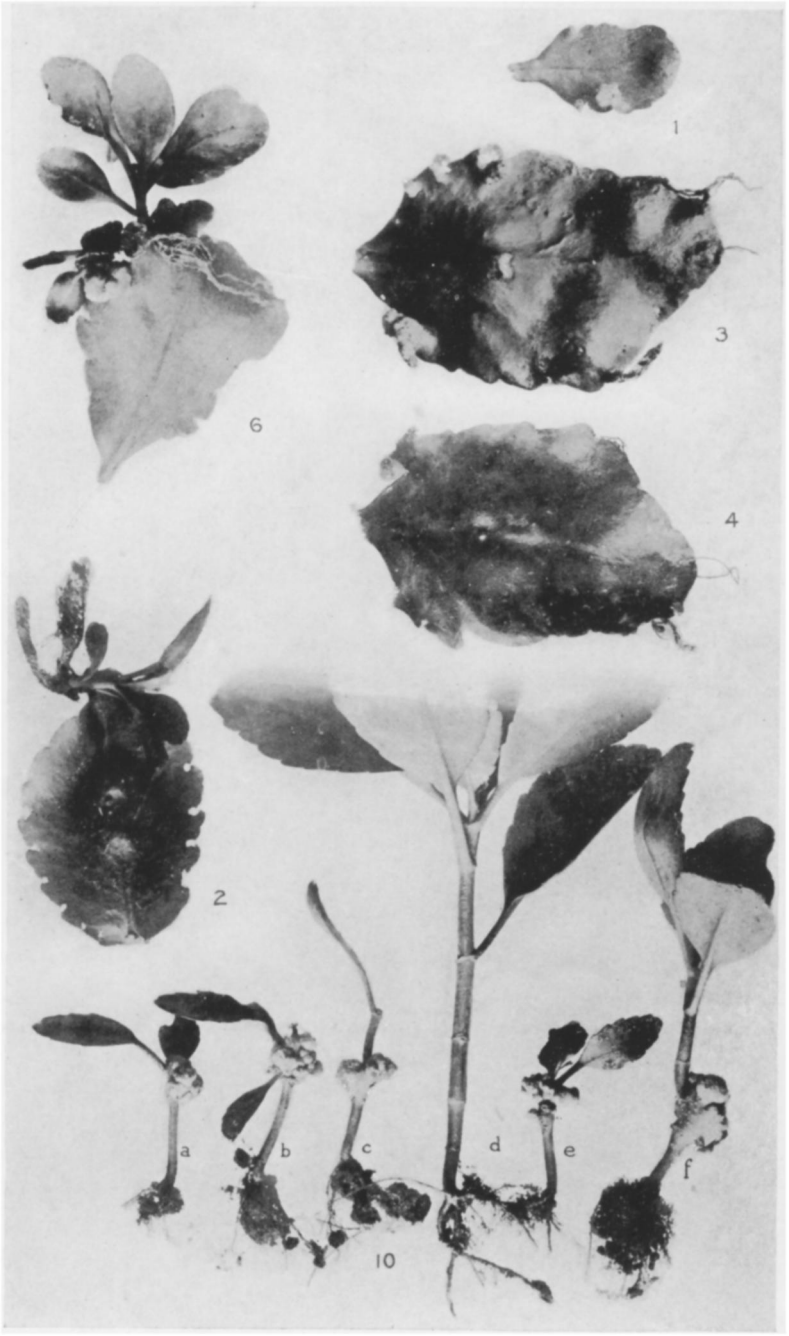
PLATE 18

FIG. 5. Old leaf, inoculated at the basal notches 140 days previously, showing a large crown gall. The control notches show normally developed plants coming from them.

FIG. 7. Old leaf, 140 days after inoculations were made near the notches, showing dwarfed leafy shoots growing in contact with crown galls. The control notches have developed normal plants.

FIG. 8. Old leaf attached to mother plant, showing a large gall on the midvein produced by inoculating it with *Bacterium tumefaciens* five months previously.

FIG. 9. Same leaf now detached, nine months after inoculation.



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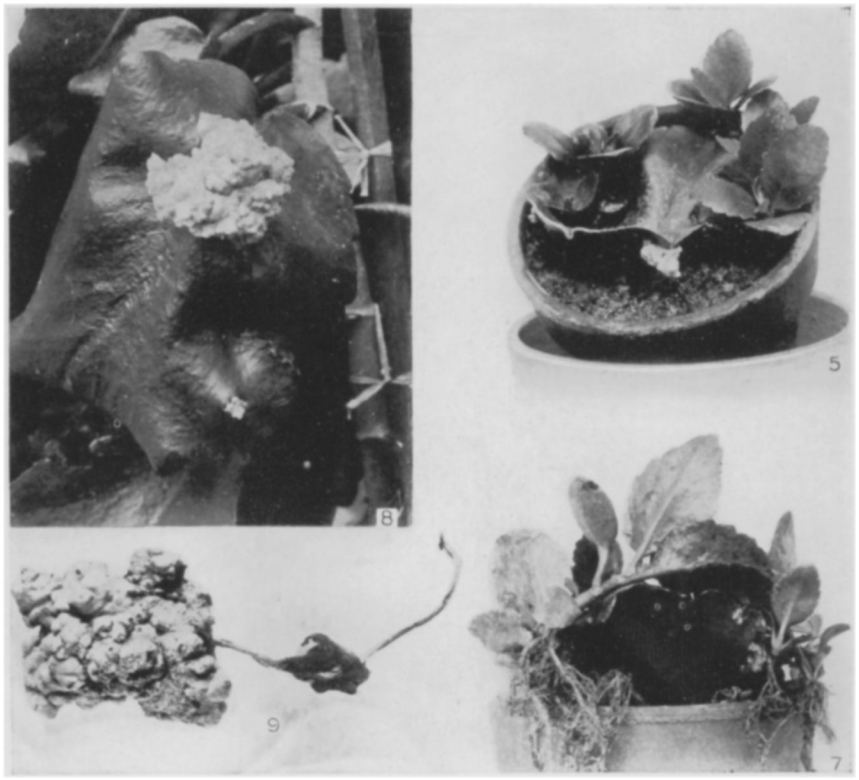

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